Reference Number 02-0359

Northern Leopard Frog (Rana pipiens) Egg Mass Survey

Prepared for:

General Electric Company

Northern Leopard Frog (Rana pipiens) Egg Mass Survey

Prepared for:
General Electric Company
Pittsfield, Massachusetts

Prepared by:
ARCADIS G&M, Inc.
Portland, Maine

Technical Advisor:
William J. Resetarits, Ph.D.
Old Dominion University
Norfolk, Virginia

Our Ref.:
ME100040.0005.00001

Date:
May 2003

Table of Contents

1. Introduction 1

2. Methods 2

2.1 Mobilization 2

2.2 Field Surveys 4

2.3 Data Analysis 5

3. Results 7

4. Discussion 10

5. References 11

Tables

3-1 Summary of Survey Observations

3-2 Incidence of Egg Masses in Ponds

3-3 Analysis of Covariance and Analysis of Variance Results

Figures

1-1 Ponds Surveyed for Egg Masses, Spring 2003, Housatonic River, Massachusetts

3-1 Proportion of Ponds with Breeding Activity (Using Ranges of tPCB Concentrations in Pond Sediment)

3-2 Density of Egg Masses (Using Ranges of tPCB Concentrations in Pond Sediment)

3-3 Density of Egg Masses (Using Average tPCB Concentrations in Pond Sediment)

Appendices

A Egg Mass Database

B Northern Leopard Frog Egg Mass Survey Photo Log

C Field Data Sheets

1. Introduction

Between the 1930s and 1977, polychlorinated biphenyls (PCBs) were used in manufacturing processes at the General Electric Company (GE) facility in Pittsfield, Massachusetts. Prior to 1977, releases of PCBs were conveyed to the Housatonic River and deposited in downstream sediments. During periodic flooding, sediments containing PCBs were deposited on the soils and in the ponds within the floodplain. Average concentrations of PCBs in pond sediments within the primary study area (PSA) of the Housatonic River – defined by the U.S. Environmental Protection Agency (EPA) as the section of the river and its floodplain from the confluence of the East and West Branches of the Housatonic River to Woods Pond Dam (Figure 1-1) – range from 0.31 mg/kg to 230 mg/kg (030703_usepa_hr_dbase1.mdb, from the data exchange agreement between EPA and GE).

Rana pipiens, the Northern leopard frog, is a medium sized ranid amphibian that is relatively common in Massachusetts and is often abundant in localized areas, although it can be locally uncommon throughout much of its range. In western Massachusetts, *R. pipiens* is found in the major drainage basins, including the Housatonic River (Kenny 2000). *R. pipiens* breed and overwinter in a variety of water bodies such as ponds, marshes, sloughs, dugouts, borrow pits, oxbows, beaver impoundments, shallow bays and lake margins, quiet backwaters of streams and rivers, slow flowing creeks, roadside ditches, and springs[1] (Kendall 2002). After breeding, adults move to grasslands or damp woody areas. *R. pipiens* larvae (i.e., tadpoles) are primarily herbivorous, but will also scavenge on dead animals (Merrell 1977). After metamorphosis, the frogs are primarily carnivorous. Adult *R. pipiens* primarily feed on insects and worms, but may also consume snails, crustaceans, spiders, and other small frogs (Merrell 1977). Given the life history of *R. pipiens*, as well as the distribution of PCBs within the floodplain of the Housatonic River, both adult and larval *R. pipiens* may be exposed to PCBs through the diet and through dermal contact. In addition to these pathways, eggs, larvae, and metamorphs may also be exposed to PCBs through maternal transfer.

In 2000, as part of EPA's ecological risk assessment of the PSA, Fort Environmental Laboratories conducted a study of the reproductive fitness of adult *R. pipiens* inhabiting the PSA of the Housatonic River floodplain (Fort 2003). Because field-

[1] For simplicity, such breeding water bodies are hereafter referred to as ponds.

collected females possessed virtually no mature oocytes, Fort (2003, p. 43) concluded that *R. pipiens* collected from the PSA "showed marked signs of reproductive stress," and therefore, "it is unlikely that female specimens collected from the target site sampling locations would have been capable of reproducing successfully in the field under natural conditions." In addition, the finding of only six egg masses in ponds located within the study area was presented as further evidence of reproductive stress (Fort 2003). However, Fort's observations may have any number of explanations, including the potential premature or post-oviposition collection of *R. pipiens* adults, the inappropriate comparison of the reproductive state of randomly collected target frogs to reference frogs from a laboratory supply company, extended holding times of target frogs prior to evaluation of reproductive fitness, and apparent inclusion of juvenile frogs in the assessment of female reproductive fitness (see BBL Sciences et al. 2003).

Given the many potential confounding factors in Fort's (2003) study, GE commissioned a breeding season survey of *R. pipiens* egg masses occurring in ponds throughout the PSA (Figure 1-1). The survey was conducted by ARCADIS G&M, Inc. (ARCADIS), with technical advice provided by William J. Resetarits, Ph.D., of Old Dominion University. The primary objective of this survey was to determine whether adult *R. pipiens* are in fact failing to reproduce successfully in the field under natural conditions, as asserted by Fort (2003). Because reproductive fitness of adult female *R. pipiens* is the most sensitive endpoint identified by Fort (2003), our survey focused on a primary indicator of reproductive fitness: oviposition.

This report presents the methods (Section 2), results (Section 3), and implications of the *R. pipiens* egg mass survey (Section 4). References are presented in Section 5.

2. Methods

During the 2003 breeding season (April 21 through May 8), ARCADIS surveyed ponds throughout the PSA for *R. pipiens* egg masses. Surveys for egg masses were conducted in appropriate breeding habitat, including vernal pools, ponds, backwaters, and marshes of the Housatonic River. The following subsections detail the methodology employed in the survey, including mobilization, field surveying, and data analysis.

2.1 Mobilization

Mobilization required before the initiation of the field survey included field reconnaissance, as well as ambient air and surface water temperature monitoring. The

literature on the life history of *R. pipiens* (Wright 1920; Hine, Les et al. 1981; Kendall 2001) reports that breeding begins when air and water temperatures reach 10 degrees Celsius (C); therefore, the goal of early air and water temperature monitoring was to identify a start date for the surveys in advance of the initiation of breeding. Towards that end, beginning on March 19, 2003, ponds within the floodplain were visited and surface water temperatures were recorded using a self-calibrating field multimeter (Hanna Combo Tester Low Range, Model 89306, Woonsocket, RI).

Additionally, between April 17, 2003 and April 20, 2003, field biologists walked throughout the PSA in order to identify suitable breeding habitat for *R. pipiens* and suitable microhabitat for the deposition of egg masses. Reconnaissance activities targeted ponds in the study area known to have produced *R. pipiens* eggs in the past (e.g., EPA 8-VP-1, EPA 8-VP-2/BBL E-5, EPA 38-VP-2, EPA 46-VP-5, and EPA W-4/BBL 13.6)[2], as well as other ponds in the study area that met the following criteria describing suitable breeding habitat (Kendall 2002):

- Some degree of permanence (i.e., unlikely to dry up before tadpole metamorphosis);
- Abundant aquatic and emergent vegetation, such as cattails, sedges, rushes, and moisture tolerant grasses that provide cover and a substrate for egg mass deposition;
- Shallow open water that receives direct sunlight in the early morning and afternoon, often on the north side of the pond;
- Standing water that freezes solid during most winters or that may dry up every few years, thereby reducing or preventing the establishment of populations of predatory fish (Merrell and Rodell 1968);
- Non-acidic water with a pH between 6.5 and 8.5 (Nace, Culley et al. 1996);
- Water depth between 10 and 65 cm (Gilbert, Leclair et al. 1994); and

---

[2] To the extent that both EPA and BBL designated pond identification numbers, both designations are presented in this report, using the format EPA ___/BBL ___. However, EPA ponds 8-VP-1, 38-VP-2, and 46-VP-5 do not appear to have BBL designations.

- Gradually sloping shoreline to support emergent and adjacent upland vegetation (Wershler 1991).

2.2 Field Surveys

Field surveys were conducted on 15 days between April 21, 2003 and May 8, 2003. The initiation date was based on surface water temperatures (as discussed above), while the end date was based on the timing of egg hatching. Surveys were conducted between approximately 8:00 am and 6:00 pm to take advantage of optimal light conditions.

Prior to surveying each pond, general information on field conditions and pond habitat was recorded on the field data form. Information recorded included: geographic positioning system (GPS) location, recent rainfall amounts (measured using a rain gauge located within the PSA), pond length and width, weather conditions, survey start time, end time and duration. Specific information about each pond was recorded, including: water body type (e.g., lake/pond, marsh, bog/fen etc.), water flow, water depth, bottom substrate, percent area of pond surface with vegetative cover, types of vegetation in the pond, water color, water clarity, origin (natural/constructed), and permanence. The determination of permanence was based on professional judgment in the field, considering factors such as: pond depth and size, types of vegetation (e.g., cattails, loosestrife), changes in pond area during the course of the survey period, and recent historical observations of pond persistence. Water temperature and pH were measured at the approximate center of the pond using a self-calibrating field multimeter (Hanna Combo Tester Low Range, Model 89306, Woonsocket, RI).

R. pipiens egg masses were identified by two observers based on appearance and timing. Frog egg masses are easily distinguished from salamander egg masses (which are smaller, have fewer larger eggs, and have a stiff gelatinous clear or white matrix) and toad egg masses (which are laid in strings). Spring peepers (*Pseudacris crucifer*), which lay scattered single eggs, are also readily distinguishable, while "the yellow or gold-colored embryos of pickerel frogs are quite striking and cannot be confused with other frogs" (Kenney and Burne 2000). Hence, only egg masses of *R. sylvatica,* which lay eggs at approximately the same time as *R. pipiens*, could be confused with egg masses of *R. pipiens*. However, *R. pipiens* lay egg masses individually, while *R. sylvatica* lay egg masses in communal aggregations (Wright and Wright 1949; Seale 1982; Corn and Livo 1989; Crouch and Paton 2000). Egg masses of *R. pipiens* are grapefruit-sized (8 to 13 cm in diameter) and irregularly globular, while egg masses of *R. sylvatica* are plum- or orange-sized (5 to 7 cm in diameter) and globular (Corkran

and Thoms 1996). Additionally, egg masses of *R. pipiens* are less gelatinous than those of *R. sylvatica*. *R. sylvatica* egg masses also have a wider spacing of egg centers, compared with egg masses of *R. pipiens*. In the event that the species of an egg mass could not be unambiguously confirmed by both of the qualified observers present, it was not included in the survey; as a result, overall *R. pipiens* egg mass counts provide a conservative estimate of the actual number in the PSA.

During each survey, observers walked slowly throughout the pond in waders, taking care not to disturb the egg masses unnecessarily. Upon encountering an *R. pipiens* egg mass, its location was marked with flagging, it was photographed, and descriptive information was recorded. Each egg mass was assigned an identification number. A plastic ruler was included in the photograph for scaling purposes. Eggs observed during the study were qualitatively classified as to their approximate developmental stage using a modification of Gosner (1960). Because development proceeds rapidly after fertilization, Gosner stages 1 to 7 were not observed during the study. Gosner stages 7 through 16 were referred to as blastula/gastrula stage and Gosner stages 17 through 20 were referred to as tail bud forms. Later in the study, tadpole hatching was recorded, if observed. According to Gosner (1960), embryos of most species hatch between stages 17 and 20. The documentation of approximate developmental stage provided an indication that eggs had been fertilized and embryos were developing.

Four ponds (BBL 3.3B, EPA 54-VP-1/BBL 15.9, 46-VP-2/ BBL 12.5, and BBL 12.2) were not surveyed either due to a lack of suitable habitat or for reasons of personal safety (i.e., very muddy substrates or dense vegetation).

2.3 Data Analysis

Data analysis depended in part on historical measurements of concentrations of PCBs in the sediment of each pond. Pond sediment samples were collected by Weston Solutions, Inc., as part of surveys conducted in 1998 and 1999 on behalf of EPA. The pond sediment sampling and analytical methods employed are detailed in GE's recent RCRA Facility Investigation (RFI) Report (BBL and QEA 2003). The data used were obtained through the data exchange agreement between EPA and GE (030703_usepa_hr_dbase1.mdb). The arithmetic mean concentration of total PCBs in the top six inches of sediment in each pond was calculated and used as the representative measure of exposure for each pond. For pond BBL 4.7[3], there were two

[3] The pond designated by BBL as 4.7 does not appear to have a number designated by EPA.

PCB samples with inconsistent sampling information. In one case, a sample with Geographic Information System (GIS) coordinates just outside of the areal extent of the pond was listed as a sediment sample (FL000467). This sample was included as a pond sediment sample, under the assumption that the samplers were unlikely to misclassify the environmental medium, whereas the accuracy of GPSs may vary by one meter or more. In the other case, a sample with soil matrix had GIS coordinates that placed it within EPA pond 4.7 (FL002020). Using the logic described above, this sample was not included as a pond sediment sample.

At the completion of the field surveys, data from the field data sheets were entered into a Microsoft Excel spreadsheet. Dependent variables were defined as the presence or absence of egg masses and the total number of *R. pipiens* egg masses per pond. The independent variables were defined as the arithmetic average concentrations of total PCBs in pond sediment (mg/kg) and pond area (m^2).

The possible relationship between concentrations of PCBs in pond sediment and reproductive fitness of *R. pipiens* was tested in three ways. First, the Chi-squared (X^2) test was used to examine the possible relationship between sediment PCB concentration and the incidence of egg masses in the surveyed ponds (i.e., presence/absence). For the X^2 test, average concentrations of PCBs in sediment were classified into four ranges, divided in a manner that yielded similar sample sizes per group -- i.e., less than 5 mg/kg, 5 to 20 mg/kg, 20 to 40 mg/kg, and greater than 40 mg/kg.

Second, the potential relationship between PCBs in pond sediment and the numbers of egg masses was explored using Analysis of Covariance (ANCOVA)/Analysis of Variance (ANOVA), as well as the Student-Newman-Keuls Multiple comparisons test. ANCOVA was used to deconvolute the potential influence of pond area on any relationship between concentrations of PCBs in sediment and egg mass numbers. This is a better approach than analyzing density per se, because of the pitfalls inherent in analyses of ratios (e.g., number of egg masses/area of pond) and because pond area is a relatively poor measure of available habitat.[4] The ANCOVA tests first for an effect of area on the number of egg masses and removes any variation due to area before testing the effects of PCBs. We also used the Student-Newman-Keuls Multiple comparisons test to account for differences in the numbers of egg masses in each range of PCB concentrations. This ensures the conservatism of the analysis. Since the covariate

---

[4] In large ponds, the majority of the pond bottom area is typically unsuitable breeding habitat for *R. pipiens*.

(area of pond) in the ANCOVA had no significant effect, we ran an ANOVA to test the significance of the main effect (concentration of PCBs), yielding a potentially more powerful statistical test through the retention of more degrees of freedom in the F-ratio.

Third, multiple linear regression was used to explore the possible relationship between the average concentration of sediment PCBs, area, and the number of egg masses in ponds with breeding activity.[5] For this analysis, the number of egg masses was modeled against pond area and the average concentration of PCBs in pond sediment. As described above, this approach avoids the statistical problems associated with analyzing density.

3. Results

Forty-four ponds within the PSA of the Housatonic River floodplain were surveyed for *R. pipiens* eggs masses. During the fifteen-day survey period, 216 *R. pipiens* egg masses were identified and documented in 17 of those ponds. Table 3-1 summarizes the egg mass survey observations. The complete database is provided in Appendix A, while a photographic log is provided in Appendix B, and copies of the field data sheets are provided in Appendix C.

Average concentrations of total PCBs in sediment of those 44 ponds range from 0.46 mg/kg to 230 mg/kg. Because average concentrations of total PCBs in sediment in all PSA floodplain ponds range from 0.31 mg/kg to 230 mg/kg, the surveyed ponds represent a range that includes the vast majority of PCB concentrations in potential *R. pipiens* breeding ponds throughout the PSA. The physical characteristics of the ponds surveyed also span the range of expected suitable breeding habitats for *R. pipiens* (Table 3-1). Of the 44 ponds surveyed, 19 are permanent and the rest are temporary. Most have a silt/mud bottom substrate, although two have sand and gravel and two have a mixture of silt, mud, sand, and gravel. At the time of the surveys, 23 of the ponds were clear in color and the rest were stained with tannic material. Thirteen ponds were cloudy (i.e., turbid) and the remaining 31 ponds were clear. Only three ponds had no emergent vegetation. Two ponds had pH less than 6.5, while the other 42 ponds had pH between 6.5 and 8.5. Seven ponds had surface water temperatures

---

[5] The multiple linear regression did not include ponds with no egg masses for two reasons: (1) as discussed below, the X^2 analysis showed no evidence of a relationship between presence/absence of egg masses and PCB concentrations in pond sediment; and (2) it is not logical to conduct a regression with more than half of the values being zero.

equal to or less than 10 degrees C, while the remaining 37 ponds had surface water temperatures greater than 10 degrees C at the time of the survey. Adult *R. pipiens* were observed in 16 ponds, based on visual observations and/or calls. Most ponds (i.e., 25) had signs of other amphibian species, including adults and/or egg masses. Fish were observed in seven of the surveyed ponds. For a summary of these pond characteristics, see Table 3-1.

Table 3-2 presents the results of X^2 analysis for the proportion of ponds with egg masses, based on the four ranges of concentrations. There were no significant differences in egg mass incidence across the four ranges of concentrations of PCBs in pond sediment ($X^2_3 = 1.4$; $p = 0.70$). Figure 3-1 illustrates egg mass incidence across the four ranges of concentrations of PCBs in pond sediment.

The ANCOVA results are presented in Table 3-3. These results show that there was no significant relationship between area and number of egg masses or between PCB concentration (corrected for area) and number of egg masses ($F_{3,39} = 0.41$; $p = 0.75$). To further test the potential influence of sediment PCB concentrations on the number of egg masses, we performed the Student-Newman-Keuls Multiple comparisons test on the means for each of the four concentration ranges. These comparisons yielded no significant differences at the alpha (p) = 0.05 level. Because the area effect was not significant, we also conducted the analysis without the covariate (i.e., ANOVA), again finding no significant relationship ($F_{3,40} = 0.52$; $p = 0.67$) (Table 3-3.). Figure 3-2 illustrates the densities of egg masses across all ponds and for each of the four PCB ranges used in the ANCOVA/ANOVA analysis. None of these analyses provides any evidence of an effect of PCB concentrations in pond sediment on the number of egg masses in the ponds.

Multiple linear regression was used to evaluate the relationship between the number of egg masses per pond and both the pond area and the concentration of PCBs in pond sediment (i.e., without segregating ponds into four ranges of concentrations). The R^2 from this analysis was 0.046, indicating that pond area and concentration of PCBs in sediment together accounted for only 4.6 percent of the variability in the number of egg masses per pond. The slope term for pond area was not significantly different from zero ($F_{1,14} = 0.047$, $p = 0.83$). Similarly, the slope term for sediment PCB concentration was not significantly different from zero ($F_{1,14} = 0.457$, $p = 0.51$). The results of the multiple linear regression thus provide no evidence of a PCB effect on the number of egg masses per ponds. With respect to density, Figure 3-3 illustrates the absence of a relationship between egg mass density and the concentration of PCBs in pond sediment.

During the course of the field study, development of embryos within the egg masses and the presence of *R. pipiens* larvae in the ponds were noted (Appendix A, Table A-2). In only one pond with egg masses (EPA 19-VP-5/ BBL 4.6, PCBs 50 mg/kg) was the survey team unable to unambiguously identify any fertilized eggs (as determined by observations of eggs reaching the blastula/gastrula stage). Of the remaining ponds with egg masses, there were only two ponds where none of the egg masses appeared to be obviously progressing towards hatching (EPA 40-VP-3/ BBL 10.7, PCBs 4.7 mg/kg; EPA 8-VP-1, PCBs 9.1 mg/kg), as measured by the presence of eggs beyond the blastula/gastrula stage. It is important to note that the absence of eggs beyond the blastula/gastrula stage does not mean that the eggs did not develop, but rather that they had not progressed at the time they were surveyed.

As evidenced by the presence of hatched tadpoles within *R. pipiens* egg masses, *R. pipiens* larvae were observed in the following ponds: EPA pond 8-VP-4/BBL pond 1.2, EPA pond 23A-VP-1/BBL pond 6.4, EPA pond 42-VP-1, EPA pond 49A-VP-1/BBL pond 15.5, EPA pond 42-VP-3/BBL pond 11.1, EPA pond 42-VP-4, and EPA pond 42-VP-5.[6] Because these observations were incidental, the converse is not necessarily true (i.e., we cannot say that no larvae were present in the other ponds). The average concentration of PCBs in pond sediment in those ponds where *R. pipiens* larvae were observed ranged from 0.46 mg/kg to 76.4 mg/kg. Hence, egg masses deposited in these ponds were capable of producing live larvae. Because surveys concluded prior to metamorphosis, no qualitative observations can be made regarding metamorphosis.

The information on habitat variables provided by the survey was not quantitatively evaluated because none of these is directly germane to the issue of possible PCB effects on *R. pipiens* reproduction. They are generally qualitative measures, and were collected to provide a background context for evaluating the study and any observed relationships to PCBs. It is worth noting, however, that the two ponds with pH outside of the range of 6.5 to 8.5 (i.e., EPA pond 46-VP-4/BBL pond 13.11 with a pH of 5.95 and EPA pond 46-VP-1/BBL pond 12.6 with a pH of 6.34) both contained zero egg masses, as predicted by Nace, Culley et al. (1996). Similarly, with one exception, all ponds with water temperatures at or below 10 degrees C (n = 7) contained zero egg masses, as predicted by Wright (1920), Hine, Les et al. (1981), and Kendall (2001). Finally, consistent with the findings of Kendall (2002), egg masses were more often present in ponds judged permanent than in ponds judged temporary.

---

[6] EPA ponds 42-VP-1, 42-VP-4, and 42-VP-5 do not appear to have BBL designations.

4. Discussion

The 2003 survey of *R. pipiens* egg masses in 44 ponds throughout the floodplain of the PSA showed no evidence of reproductive impairment in terms of the ability of females to produce eggs capable of being fertilized and the ability of males to fertilize them. A total of 216 *R. Pipiens* egg masses were observed in 17 of the 44 ponds that were surveyed. In many instances, we were able to confirm that these eggs were fertile and successfully produced living larvae. Additionally, there was no evidence of a relationship between concentrations of total PCBs and number or incidence of *R. pipiens* egg masses. The ponds surveyed represented a broad range of habitat characteristics and concentrations of total PCBs in sediment, both of which were representative of the overall spectrum of potential breeding ponds in the floodplain. Hence, it is reasonable to extrapolate from these ponds to the entire PSA and to conclude that there is no evidence of the reproductive impairment in *R. pipiens* that was suggested by Fort (2003). The striking difference between the numbers of egg masses found in the present study (n = 216) and the numbers reported by Fort (2003) (n = 6) suggests that some factor not related to sediment PCB concentrations most likely accounted for the low numbers of egg masses reported by Fort. Similarly, the presence of fertilized egg masses in the ponds in 2003 demonstrates the female *R. pipiens* inhabiting the PSA were capable of producing mature oocytes and that males were capable of fertilizing them. This finding further suggests that one or more confounding factors influenced the outcome of the Fort (2003) study.

5. References

BBL and QEA. 2003. Housatonic River – Rest of River RCRA Facility Investigation Report. Vol. 1. Report. General Electric Company, Pittsfield, Massachusetts. Prepared by Blasland, Bouck & Lee, Inc., Syracuse, New York, and Quantitative Environmental Analysis, Inc., Liverpool, New York. January.

BBL Sciences, Branton Environmental Consulting, and W. J. Resetarits. 2003. Comments on the Final Report – Frog Reproduction and Development Study: 2000 Rana pipiens Reproduction and Development Study. Prepared by BBL Sciences, Long Beach, California, Branton Environmental Consulting, Long Beach, California, and Dr. William J. Resetarits, Old Dominion University, Norfolk, Virginia. February.

Corkran, C. C., and C. R. Thoms. 1996. Amphibians of Oregon, Washington and British Columbia. Lone Pine Publishing, Quality Colour Press, Edmonton, AB, Canada. 175 pp.

Corn, P. S., and L. J. Livo. 1989. Leopard Frog and Wood Frog reproduction in Colorado and Wyoming. Northwest. Nature. 70:1-9.

Crouch, W.B., and P.W.C. Paton. 2000. Using egg-mass counts to monitor wood frog populations. Wildlife Society Bulletin 28:895-901.

Fort Environmental Laboratories, Inc. (Fort). 2003. Final Report – Frog Reproduction and Development Study, 2000 Rana pipiens Reproduction and Development Study. Prepared by Fort Environmental Laboratories, Inc., Stillwater, OK for Weston Solutions, Inc., West Chester, PA. January.

Gilbert, M., R. LeClair, and R. Fortin. 1994. Reproduction of the northern leopard frog (*Rana pipiens*) in floodplain habitat in the Richelieu River, P. Quebec, Canada. Journal of Herpetology 28:465-470.

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 1960:183-190.

Hine, R.L., Les, B.L., and Hellmich, B.F. 1981. Leopard frog populations and mortality in Wisconsin, 1974-76. Technical Bulletin No. 122, Department of Natural Resources, Madison, Wisconsin. 39 pp.

Kendell, K. 2001. Northern leopard frog reintroduction: Raven River – year 2 (2000). Alberta Species at Risk Report No. 13. Alberta Sustainable Resource Development, Fish and Wildlife Service, Edmonton, Alberta, Canada. 43 pp.

Kendell, K. 2002. Survey protocol for the northern leopard frog. Alberta Species at Risk Report No. 43. Alberta Sustainable Resource Development, Fish & Wildlife Division, Resource Status and Assessment Branch, Edmonton, Alberta, Canada. 30 pp.

Kenney, L.P., and M.R. Burne. 2000. A field guide to the animals of vernal pools. Massachusetts Division of Fisheries and Wildlife, Natural Heritage and Endangered Species Program and Vernal Pool Association. Westborough, MA.

Merrell, D.J. 1977. Life history of the leopard frog, *Rana pipiens*, in Minnesota. Occupation Paper No. 15. University of Minnesota Bell Museum of Natural History. 23 pp.

Merrell, D.J., and C.F. Rodell. 1968. A comparison of the estimated size and the "effective size" of breeding populations of the leopard frogs, Rana pipiens. Evolution 22:274-283.

Nace, G.W., D.D. Culley, M.B. Emmons, E.L. Gibbs, V.H. Hutchison, and R.G. Mckinnell. 1996. Amphibians: Guidelines for the breeding, care and management of laboratory animals. A report of the Subcommittee on Amphibian Standards, Committee on Standards, Institute of Laboratory Animal Resources and National Research Council, National Academy of Sciences, Washington, DC.

Seale, D. 1982. Physical factors influencing oviposition by the Woodfrog, Rana sylvatica, in Pennsylvania. Copeia 1982(3): 627-635.

Wershler, C. 1991. Status of the northern leopard frog in Alberta – 1990. Alberta Forestry Lands and Wildlife. Unpublished Report, Edmonton, Alberta, Canada.

Wright, A.H. 1920. Frogs: their natural history and utilization. Bureau of Fisheries, document No. 888. Washington Government Printing Office, Washington, DC. 44 pp.

Wright, A.H., and A.A. Wright. 1949. Handbook of Frogs and Toads of the United States and Canada. Comstock Publ. Co., Ithaca, NY.

Table 3-1. Summary of Survey Observations

EPA Pond Identification Number	BBL Pond Identification Number	Number of Egg Masses	Survey Date	Water Body Type [a]	Average Concentration of tPCBs in Sediment (mg/kg)	Calculated Pond Area (m^2)	Egg Mass Density (per m^2)	Air Temperature (C)	Water Temperature (C)	pH	Notes
W-4	13.6	26	4/24/2003	LP,M,BP	0.46	7,854	3.3E-03	9.1	8.7	7.41	b
W-4 (E)	13.6 (E)	0	5/5/2003	B	0.46	2,749	0.0E+00	18.9	18.6	7.39	c
8-VP-4	1.2	2	5/4/2003	LP	0.47	1,414	1.4E-03	18.1	14.1	7.58	
46-VP-4	13.11	0	5/5/2003	LP	0.53	12	0.0E+00	17.4	14.6	5.95	
46-VP-1	12.6	0	5/5/2003	LP, BP	0.76	962	0.0E+00	18.3	14.4	6.34	
23A-VP-1	6.4	2	5/7/2003	M,BP	0.90	11,781	1.7E-04	18.7	15.8	7.82	
18-VP-2	3.1	0	5/7/2003	LP	1.1	2,337	0.0E+00	22.2	19.7	7.68	
46-VP-5	none	0	4/24/2003	LP	1.1	628	0.0E+00	3.6	5.6	7.42	
18-VP-1	3.2	0	5/7/2003	LP	1.7	982	0.0E+00	20.1	18.3	7.71	
27-VP-1	7.9	20	4/30/2003	LP	1.7	4,595	4.4E-03	20.7	20.4	7.64	
27-VP-1 (W)	7.9 (W)	4	5/1/2003	LP	1.7	2,592	1.5E-03	14.8	14.1	7.15	d
none	6.3	0	5/5/2003	LP	2.0	1,571	0.0E+00	19.2	18.1	7.75	
27B-VP-2	7.1	0	4/30/2003	LP	4.1	3,181	0.0E+00	16.9	13.4	7.32	
40-VP-1	10.9	11	4/21/2003	LP	4.7	825	1.3E-02	22.6	20.9	8.13	
40-VP-3	10.7	5	4/22/2003	LP	4.7	1,178	4.2E-03	12.5	12.4	7.14	
42-VP-1	none	15	5/1/2003	LP	7.5	2,474	6.1E-03	22.7	20.1	7.62	
58-A-VP-1	17.5	0	4/25/2003	M,B	8.4	3,181	0.0E+00	8.3	6	7.17	
8-VP-1	none	1	4/23/2003	LP	9.1	236	4.2E-03	6.9	10.1	7.51	
27B-VP-3	none	0	4/30/2003	LP	10	648	0.0E+00	17	14.4	7.19	
33-VP-1	8.1	0	5/8/2003	LP	10	785	0.0E+00	14.1	14.1	7.9	
19-VP-6	5.1	55	5/3/2003	LP	12	3,927	1.4E-02	18.9	20.3	8.13	
27-VP-2	7.3	0	4/30/2003	LP	14	1,178	0.0E+00	15.8	14.6	7.35	
49A-VP-1	0	0	5/6/2003	LP, B	15	236	0.0E+00	15.8	12.7	7.83	
42A-VP-1	11.2	0	5/6/2003	LP	16	3,436	0.0E+00	14.4	13.5	7.81	
42-VP-3	11.1	0	5/2/2003	LP	19	589	0.0E+00	19.2	17.6	8.06	
23-VP-2	6.1	0	4/23/2003	LP	19	1,885	0.0E+00	4.8	9.7	7.79	

Notes appear on p. 6 of 6.

Table 3-1. Summary of Survey Observations

EPA Pond Identification Number	BBL Pond Identification Number	Number of Egg Masses	Survey Date	Water Body Type [a]	Average Concentration of tPCBs in Sediment (mg/kg)	Calculated Pond Area (m^2)	Egg Mass Density (per m^2)	Air Temperature (C)	Water Temperature (C)	pH	Notes
23-VP-2 (N)	6.1 (N)	0	4/23/2003	LP	19	864	0.0E+00	6.9	9.3	7.27	e
40-VP-4	10.5	18	4/22/2003	LP	23	2,003	9.0E-03	14	14.1	7.52	
40-VP-2	10.4	31	4/21/2003	LP	23	550	5.6E-02	17.1	18.1	7.35	
49-VP-1	15.7	0	5/6/2003	B	24	3,436	0.0E+00	16.5	13.6	8.11	
38-VP-1	9.4	0	4/29/2003	LP	29	3,770	0.0E+00	21.4	16.9	7.44	
38-VP-2(W)	none	8	4/25/2003	LP,B	21	4,712	1.7E-03	12.8	13.5	7.59	
38-VP-2	none	0	4/29/2003	LP	32	2,356	0.0E+00	20.2	15.1	7.56	
42-VP-2	none	0	5/2/2003	LP	39	648	0.0E+00	21.3	17.6	7.69	
23-VP-1	6.2	0	5/5/2003	LP	45	98	0.0E+00	20.8	17	7.74	
38-VP-3	W7-a	0	4/29/2003	LP	47	1,963	0.0E+00	21.6	16.5	7.45	
42-VP-4	none	7	5/2/2003	LP	48	1,257	5.6E-03	16.9	17.8	7.92	
19-VP-5	4.6	2	4/23/2003	LP	50	1,178	1.7E-03	7.9	11.3	7.78	
none	9.2	1	5/8/2003	LP	60	1,767	5.7E-04	13	14.6	8.2	
8-VP-2	E-5	0	4/23/2003	LP	61	2,199	0.0E+00	10.1	10.5	7.61	
40A-VP-1	10.6	0	5/3/2003	LP	66	236	0.0E+00	18.5	18.3	7.85	
42-VP-5	none	8	5/2/2003	LP	76	1,571	5.1E-03	11.7	14.1	7.79	
19-VP-8	4.4	0	5/3/2003	LP	105	236	0.0E+00	17.8	21.5	8.47	
none	4.7	0	5/7/2003	LP	230	785	0.0E+00	20.6	18.1	7.74	

Notes appear on p. 6 of 6.

Table 3-1. Summary of Survey Observations

EPA Pond Identification Number	BBL Pond Identification Number	Number of Egg Masses	Permanent or Temporary	Substrate	Depth (m)	Water Color	Water Clarity	Percent Vegetative Cover	Sign of Adult *R. pipiens*
W-4	13.6	26	Permanent	Silt/Mud	1-2	Clear	Clear	26-50	Yes
W-4 (E)	13.6 (E)	0	Permanent	Silt/Mud	1-2	Clear	Cloudy	1-25	No
8-VP-4	1.2	2	Temporary	Silt/Mud & Sand/Gravel	<1	Stained	Clear	1-25	No
46-VP-4	13.11	0	Temporary	Silt/Mud	<1	Stained	Clear	1-25	No
46-VP-1	12.6	0	Permanent	Silt/Mud	1-2	Stained	Cloudy	>50	No
23A-VP-1	6.4	2	Permanent	Silt/Mud	<1	Clear	Clear	>50	Yes
18-VP-2	3.1	0	Temporary	Silt/Mud & Sand/Gravel	<1	Stained	Clear	>50	No
46-VP-5	none	0	Temporary	Silt/Mud	<1	Stained	Clear	1-25	No
18-VP-1	3.2	0	Temporary	Silt/Mud	<1	Clear	Clear	26-50	No
27-VP-1	7.9	20	Permanent	Silt/Mud	<1	Stained	Clear	1-25	No
27-VP-1 (W)	7.9 (W)	4	Permanent	Silt/Mud	<1	Stained	Clear	1-25	No
none	6.3	0	Temporary	Silt/Mud	<1	Clear	Clear	26-50	Yes
27B-VP-2	7.1	0	Temporary	Sand/Gravel	<1	Stained	Clear	0	No
40-VP-1	10.9	11	Permanent	Silt/Mud	<1	Clear	Clear	1-25	Yes
40-VP-3	10.7	5	Permanent	Silt/Mud	<1	Stained	Clear	26-50	Yes
42-VP-1	none	15	Temporary	Silt/Mud	<1	Clear	Clear	>50	Yes
58-A-VP-1	17.5	0	Permanent	Silt/Mud	1-2	Clear	Clear	26-50	No
8-VP-1	none	1	Temporary	Silt/Mud	<1	Clear	Clear	1-25	No
27B-VP-3	none	0	Temporary	Sand/Gravel	<1	Stained	Clear	0	No
33-VP-1	8.1	0	Temporary	Silt/Mud	<1	Clear	Clear	1-25	No
19-VP-6	5.1	55	Permanent	Silt/Mud	1-2	Stained	Clear	1-25	Yes
27-VP-2	7.3	0	Temporary	Silt/Mud	<1	Stained	Clear	1-25	No
49A-VP-1	0	0	Temporary	Silt/Mud	<1	Clear	Cloudy	26-50	No
42A-VP-1	11.2	0	Permanent	Silt/Mud	1-2	Clear	Clear	26-50	Yes
42-VP-3	11.1	0	Permanent	Silt/Mud	1-2	Stained	Cloudy	0	No
23-VP-2	6.1	0	Permanent	Silt/Mud	<1	Clear	Cloudy	1-25	No

Notes appear on p. 6 of 6.

Table 3-1. Summary of Survey Observations

EPA Pond Identification Number	BBL Pond Identification Number	Number of Egg Masses	Permanent or Temporary	Substrate	Depth (m)	Water Color	Water Clarity	Percent Vegetative Cover	Sign of Adult *R. pipiens*
23-VP-2 (N)	6.1 (N)	0	Temporary	Silt/Mud	<1	Clear	Cloudy	26-50	No
40-VP-4	10.5	18	Permanent	Silt/Mud	<1	Stained	Clear	1-25	Yes
40-VP-2	10.4	31	Permanent	Silt/Mud	<1	Stained	Clear	1-25	Yes
49-VP-1	15.7	0	Permanent	Silt/Mud	>2	Clear	Cloudy	1-25	No
38-VP-1	9.4	0	Temporary	Silt/Mud	<1	Stained	Clear	1-25	No
38-VP-2(W)	none	8	Permanent	Silt/Mud	1-2	Stained	Clear	1-25	Yes
38-VP-2	none	0	Temporary	Silt/Mud	<1	Stained	Clear	1-25	No
42-VP-2	none	0	Temporary	Silt/Mud	<1	Clear	Clear	>50	Yes
23-VP-1	6.2	0	Temporary	Silt/Mud	<1	Clear	Clear	1-25	No
38-VP-3	W7-a	0	Temporary	Silt/Mud	<1	Stained	Clear	1-25	No
42-VP-4	none	7	Permanent	Silt/Mud	1-2	Clear	Clear	>50	Yes
19-VP-5	4.6	2	Temporary	Silt/Mud	1-2	Stained	Cloudy	1-25	Yes
none	9.2	1	Permanent	Silt/Mud	1-2	Clear	Cloudy	1-25	No
8-VP-2	E-5	0	Temporary	Silt/Mud	<1	Stained	Cloudy	1-25	No
40A-VP-1	10.6	0	Temporary	Silt/Mud	<1	Clear	Cloudy	1-25	No
42-VP-5	none	8	Temporary	Silt/Mud	<1	Clear	Cloudy	26-50	Yes
19-VP-8	4.4	0	Temporary	Silt/Mud	<1	Clear	Cloudy	1-25	No
none	4.7	0	Temporary	Silt/Mud	<1	Clear	Clear	>50	Yes

Notes appear on p. 6 of 6.

Table 3-1. Summary of Survey Observations

EPA Pond Identification Number	BBL Pond Identification Number	Number of Egg Masses	Confirmation of Fertilized Eggs	Progression of Eggs Beyond Blastula/Gastrula Stage	Sign of Other Amphibians	Observations of Fish in Pond
W-4	13.6	26	Yes	Yes	Yes	No
W-4 (E)	13.6 (E)	0	N/A	N/A	Yes	No
8-VP-4	1.2	2	Yes	Yes	No	No
46-VP-4	13.11	0	N/A	N/A	No	No
46-VP-1	12.6	0	N/A	N/A	No	No
23A-VP-1	6.4	2	Yes	Yes	Yes	No
18-VP-2	3.1	0	N/A	N/A	Yes	No
46-VP-5	none	0	N/A	N/A	No	No
18-VP-1	3.2	0	N/A	N/A	Yes	No
27-VP-1	7.9	20	Yes	Yes	Yes	Yes
27-VP-1 (W)	7.9 (W)	4	Yes	Yes	Yes	Yes
none	6.3	0	N/A	N/A	No	No
27B-VP-2	7.1	0	N/A	N/A	Yes	No
40-VP-1	10.9	11	Yes	Yes	Yes	Yes
40-VP-3	10.7	5	Yes	No	Yes	No
42-VP-1	none	15	Yes	Yes	Yes	No
58-A-VP-1	17.5	0	N/A	N/A	No	No
8-VP-1	none	1	Yes	No	No	No
27B-VP-3	none	0	N/A	N/A	Yes	No
33-VP-1	8.1	0	N/A	N/A	Yes	No
19-VP-6	5.1	55	Yes	Yes	Yes	Yes
27-VP-2	7.3	0	N/A	N/A	Yes	No
49A-VP-1	0	0	N/A	N/A	No	No
42A-VP-1	11.2	0	N/A	N/A	No	Yes
42-VP-3	11.1	0	N/A	N/A	No	No
23-VP-2	6.1	0	N/A	N/A	Yes	No

Notes appear on p. 6 of 6.

Table 3-1. Summary of Survey Observations

EPA Pond Identification Number	BBL Pond Identification Number	Number of Egg Masses	Confirmation of Fertilized Eggs	Progression of Eggs Beyond Blastula/Gastrula Stage	Sign of Other Amphibians	Observations of Fish in Pond
23-VP-2 (N)	6.1 (N)	0	N/A	N/A	Yes	No
40-VP-4	10.5	18	Yes	Yes	Yes	Yes
40-VP-2	10.4	31	Yes	Yes	Yes	No
49-VP-1	15.7	0	N/A	N/A	No	No
38-VP-1	9.4	0	N/A	N/A	Yes	No
38-VP-2(W)	none	8	Yes	Yes	Yes	No
38-VP-2	none	0	N/A	N/A	Yes	No
42-VP-2	none	0	N/A	N/A	No	No
23-VP-1	6.2	0	N/A	N/A	Yes	No
38-VP-3	W7-a	0	N/A	N/A	Yes	No
42-VP-4	none	7	Yes	Yes	No	No
19-VP-5	4.6	2	No	No	Yes	No
none	9.2	1	Yes	Yes	No	No
8-VP-2	E-5	0	N/A	N/A	No	No
40A-VP-1	10.6	0	N/A	N/A	Yes	No
42-VP-5	none	8	Yes	Yes	No	Yes
19-VP-8	4.4	0	N/A	N/A	Yes	No
none	4.7	0	N/A	N/A	Yes	No

Notes:
m = meters
mg/kg = milligrams per kilogram
per m^2 = per meter squared
C = celsius
tPCB = total polychlorinated biphenyls
N/A = not applicable
a. LP = Lake or Pond, B = Backwater, BP= Beaver Pond, M = Marsh
b. Pond section is located west of the railroad bridge and the PCB concentration is based on one sample from west side of bridge.
c. Pond section is located east of the railroad bridge. Due to a lack of data for that section, the PCB concentration is based on one sample from west side of bridge.
d. Pond is located just west of 27-VP-1. Due to a lack of data for that pond, the PCB concentration is based on data from pond 27-VP-1.
e. Pond is located just north of 23-VP-2. Due to a lack of data for that pond, the PCB concentration is based on data from pond 23-VP-2

Table 3-2. Incidence of Egg Masses in Ponds

Category	Number of Ponds with Masses	Total Number of Ponds	Proportion of Ponds with Egg Masses
All Ponds	17	44	0.39
<5 mg/kg tPCBs	7	15	0.47
5-20 mg/kg tPCBs	3	12	0.25
20-40 mg/kg tPCBs	3	7	0.43
>40 mg/kg tPCBs	4	10	0.40
		$X^2_3 = 1.4$	p = 0.70

Notes:
tPCBs = total polychlorinated biphenyls

Table 3-3. Analysis of Covariance and Analysis of Variance Results

Model Type	Source	df	SS	F	p	Interpretation
ANCOVA	Area	1	325	2.89	0.10	Not significant
	tPCBs	3	139	0.41	0.75	Not significant
	Error	39	4,396			
	Total (adjusted)	43	4,904			
	Total	44				
ANOVA	tPCBs	3	183	0.52	0.67	Not significant
	Error	40	4,721			
	Total (adjusted)	43	4,904			
	Total	44				

Notes:
ANCOVA= Analysis of Covariance
ANOVA = Analysis of Variance
tPCBs = total polychlorinated biphenyls
df = degrees of freedom
SS = sum of squares
F = ratio of the treatment mean square to the error mean square
p = probability of obtaining the observed value of F by chance alone

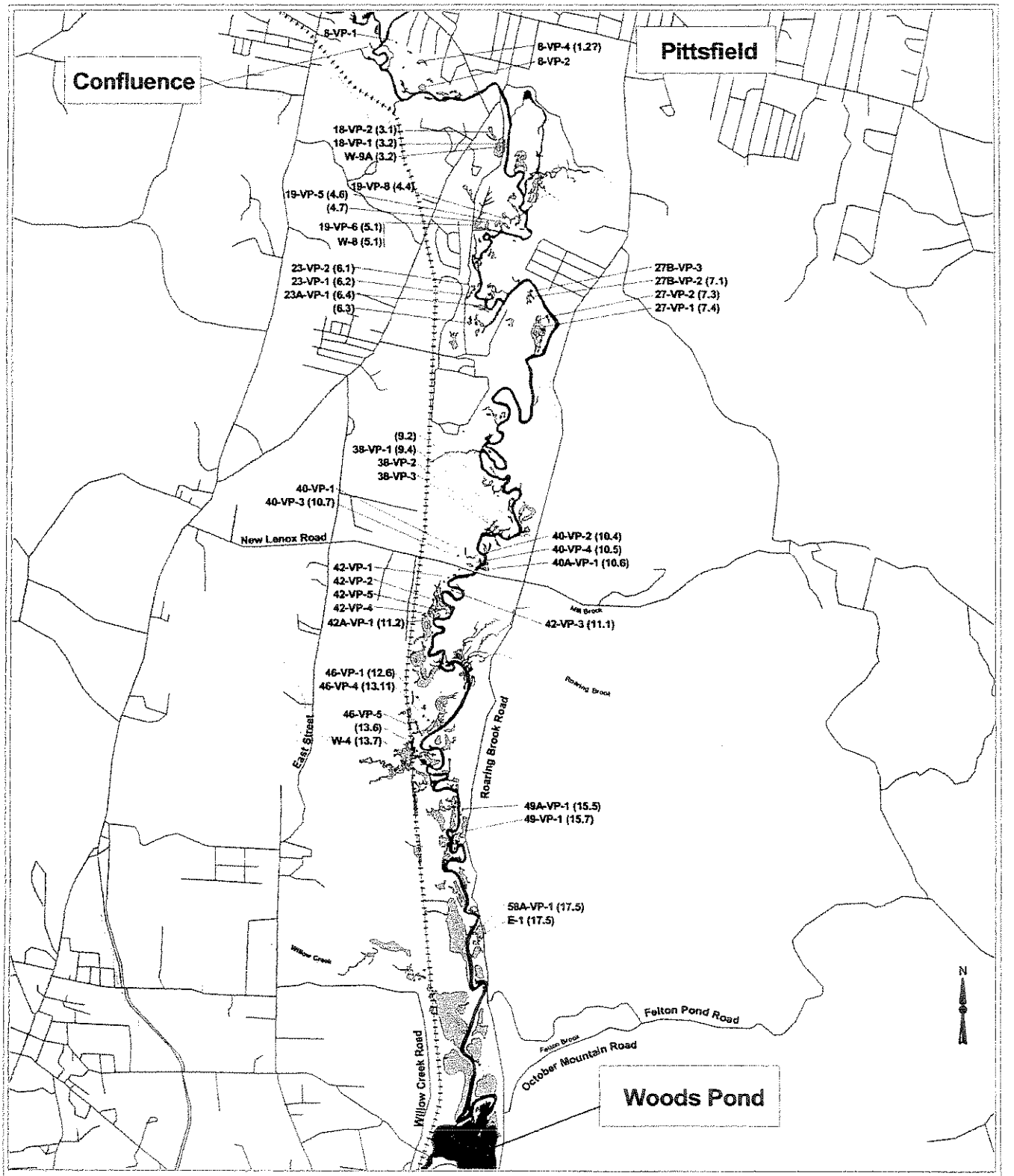


Figure 1-1

Ponds surveyed for egg masses Spring 2003

Housatonic River
Massachusetts

May 29, 2003

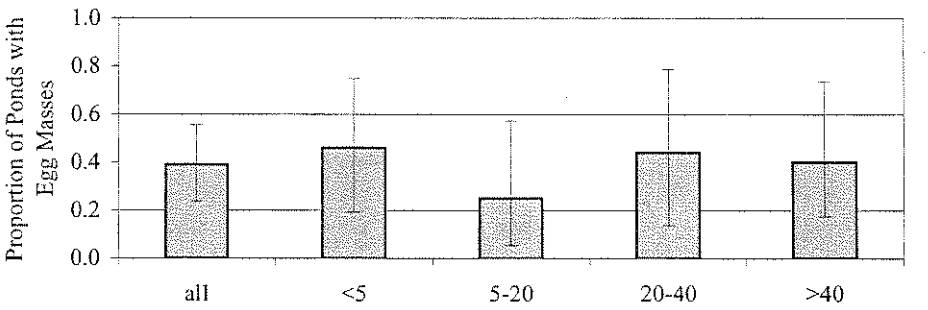
Legend

Vernal pools, ponds and backwaters

18-VP-1 (3.2)
EPA pond ID (BBL pond ID)

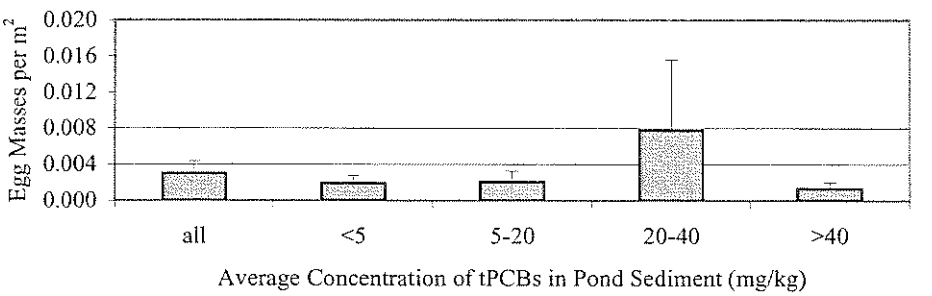
Mainstem and tributaries

Figure 3-1. Proportion of Ponds with Breeding Activity
(Using Ranges of tPCB Concentrations in Pond Sediment)



Error bars represent the 95% confidence limits of the proportion

Figure 3-2. Density of Egg Masses
(Using Ranges of tPCB Concentrations in Pond Sediment)



Error bars represent the Standard Error of the Mean

Figure 3-3. Density of Egg Masses
(Using Average tPCB Concentrations in Pond Sediment)

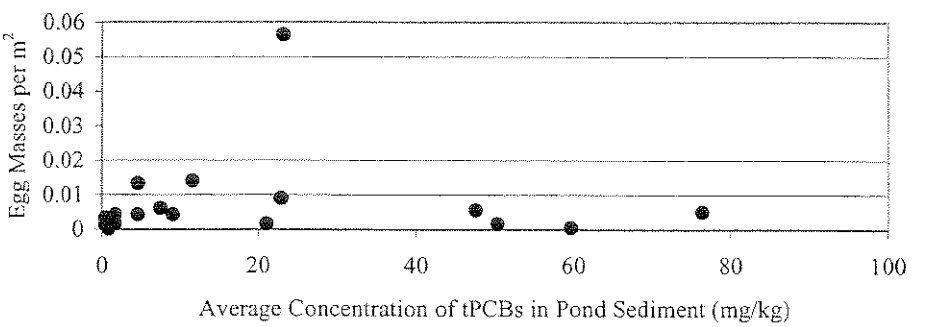


Table A-1 Survey Data

Survey Data Sheet Pond Name	EPA Pond Name	BBL Pond Name	Date	Observers [a]	Air Temp. (C)	Water Temp. (C)	pH	Recent Rain (mm)	Pond Length (m)	Pond Width (m)	Calculated Area (m^2)	Weather [b]	Wind [c]
W-4	W-4	13.6	4/24/2003	LF,TM	9.1	8.7	7.41	TRACE	125	80	7,854	PC	S
W-4 (E)	W-4 (E)	13.6 (E)	5/5/2003	KM,TM	18.9	18.6	7.39	0	50	70	2,749	OC	L
8-VP-4	8-VP-4	1.2	5/4/2003	KM,TM	18.1	14.1	7.58	0	60	30	1,414	C	C
46-VP-4	46-VP-4	13.11	5/5/2003	KM,TM	17.4	14.6	5.95	0	5	3	12	PC	L
46-VP-1	46-VP-1	12.6	5/5/2003	KM,TM	18.3	14.4	6.34	0	35	35	962	C	L
23A-VP-1	23A-VP-1	6.4	5/7/2003	KM,TM	18.7	15.8	7.82	0	150	100	11,781	C	M
18-VP-2	18-VP-2	3.1	5/7/2003	KM,TM	22.2	19.7	7.68	0	85	35	2,337	PC	C
46-VP-5	46-VP-5	none	4/24/2003	LF,TM	3.6	5.6	7.42	TRACE	40	20	628	OC	C
18-VP-1	18-VP-1	3.2	5/7/2003	KM,TM	20.1	18.3	7.71	0	50	25	982	PC	C
27-VP-1	27-VP-1	7.9	4/30/2003	KM,TM	20.7	20.4	7.64	0	90	65	4,595	C	L
27-VP-1 (W)	27-VP-1 (W)	7.9 (W)	5/1/2003	KM,TM	14.8	14.1	7.15	0	110	30	2,592	OC	L,S
6.3	none	6.3	5/5/2003	KM,TM	19.2	18.1	7.75	0	50	40	1,571	OC	L
27B-VP-2	27B-VP-2	7.1	4/30/2003	KM,TM	16.9	13.4	7.32	0	A 70, B45	A15,B20	3,181	PC	L
40-VP-1	40-VP-1	10.9	4/21/2003	LF,KM,TM	22.6	20.9	8.13	0	35	30	825	C	S
40-VP-3	40-VP-3	10.7	4/22/2003	LF,KM,TM	12.5	12.4	7.14	2.8	100	15	1,178	OC	C
42-VP-1	42-VP-1	none	5/1/2003	KM,TM	22.7	20.1	7.62	0	105	30	2,474	C	S
58-A-VP-1	58-A-VP-1	17.5	4/25/2003	LF,TM	8.3	6	7.17	0	90	45	3,181	C	C
8-VP-1	8-VP-1	none	4/23/2003	LF,KM,TM	6.9	10.1	7.51	4.6	20	15	236	OC	S
27B-VP-3	27B-VP-3	none	4/30/2003	KM,TM	17.0	14.4	7.19	0	55	15	648	PC	L
33-VP-1	33-VP-1	8.1	5/8/2003	KM,TM	14.1	14.1	7.9	0.8	50	20	785	LR	C
19-VP-6	19-VP-6	5.1	5/3/2003	KM,TM	18.9	20.3	8.13	4.2	100	50	3,927	C	L
27-VP-2	27-VP-2	7.3	4/30/2003	KM,TM	15.8	14.6	7.35	0	75	20	1,178	OC	L
49A-VP-1	49A-VP-1		5/6/2003	KM,TM	15.8	12.7	7.83	2.4	30	10	236	OC	C
42A-VP-1	42A-VP-1	11.2	5/6/2003	KM,TM	14.4	13.5	7.81	2.4	125	35	3,436	OC	L
42-VP-3	42-VP-3	11.1	5/2/2003	KM,TM	19.2	17.6	8.06	13.8	30	25	589	OC	L
23-VP-2	23-VP-2	6.1	4/23/2003	LF,KM,TM	4.8	9.7	7.79	4.6	60	40	1,885	OC	S
23-VP-2 (N)	23-VP-2 (N)	6.1 (N)	4/23/2003	LF,KM,TM	6.9	9.3	7.27	4.6	55	20	864	OC	S
40-VP-4	40-VP-4	10.5	4/22/2003	LF,KM,TM	14.0	14.1	7.52	2.8	30	85	2,003	LM	C
40-VP-2	40-VP-2	10.4	4/21/2003	LF,KM,TM	17.1	18.1	7.35	0	35	20	550	PC	S
49-VP-1	49-VP-1	15.7	5/6/2003	KM,TM	16.5	13.6	8.11	2.4	125	35	3,436	OC	L
38-VP-1	38-VP-1	9.4	4/29/2003	KM,TM	21.4	16.9	7.44	3.8	120	40	3,770	PC	C
38-VP-2(W)	38-VP-2(W)	none	4/25/2003	LF,TM	12.8	13.5	7.59	0	100	60	4,712	C	C
38-VP-2	38-VP-2	none	4/29/2003	KM,TM	20.2	15.1	7.56	3.8	100	30	2,356	C	L
42-VP-2	42-VP-2	none	5/2/2003	KM,TM	21.3	17.6	7.69	13.8	55	15	648	PC	L
23-VP-1	23-VP-1	6.2	5/5/2003	KM,TM	20.8	17	7.74	0	25	5	98	PC	S
38-VP-3	38-VP-3	W7-a	4/29/2003	KM,TM	21.6	16.5	7.45	3.8	100	25	1,963	C	L
42-VP-4	42-VP-4	none	5/2/2003	KM,TM	16.9	17.8	7.92	13.8	80	20	1,257	PC	L
19-VP-5	19-VP-5	4.6	4/23/2003	LF,KM,TM	7.9	11.3	7.78	4.6	100	15	1,178	OC	S
9.2	none	9.2	5/8/2003	KM,TM	13.0	14.6	8.2	0.8	90	25	1,767	OC	L
8-VP-2	8-VP-2	E-5	4/23/2003	LF,KM,TM	10.1	10.5	7.61	4.6	35	80	2,199	OC	S
40A-VP-1	40A-VP-1	10.6	5/3/2003	KM,TM	18.5	18.3	7.85	4.2	20	15	236	C	L
42-VP-5	42-VP-5	none	5/2/2003	KM,TM	11.7	14.1	7.79	13.8	40	50	1,571	OC	C
19-VP-8	19-VP-8	4.4	5/3/2003	KM,TM	17.8	21.5	8.47	4.2	30	10	236	C	L
4.7	none	4.7	5/7/2003	KM,TM	20.6	18.1	7.74	0	50	20	785	OC	L

Notes:
a. TM = Tom McClenahan, KM = Kelly McKay, LF = Lance Fontenot
b. C = Cloudy, PC = Partly Cloudy, OC = Overcast, LM = Lite Mist, LR = Light Rain, R = Rain
c. C = Calm, L = Light, S = Strong

Table A-1 Survey Data

Survey Data Sheet Pond Name	Geographic Positioning System, North Coordinates	Geographic Positioning System, West Coordinates	Start Time	Stop Time	Duration (min)	Water Body Type [d]	Persistance (Permanent / Temporary)	Flow [e]	Depth (m)	Substrate [f]	Vegetative Cover (%)	Emgergent Vegetative Species	Water Color [g]
W-4	42.22.749	73.14.785	12:12	17:34	5:22	LP,M,BP	Permanent	S	1-2	SM	26-50	GRASSES(ELODEA),PURPPLE LUSTREA	C
W-4 (E)	42.22.749	73.14.785	13:11	14:14	1:03	B	Permanent	S	1-2	SM	1-25	OSIER, SUB GRASSES	C
8-VP-4	42.25.946	73.14.837	8:55	10:03	1:08	LP	Temporary	N	<1	SM,SG	1-25	OSIER, LUSTRIEF	S
46-VP-4	42.22.951	73.14.699	12:00	12:02	:02	LP	Temporary	N	<1	SM	1-25	RED OSIER DOGWOOD	S
46-VP-1	42.23.016	73.14.715	10:04	11:38	:34	LP, BP	Permanent	S	1-2	SM	>50	BITTON BUSH	S
23A-VP-1	42.24.794	73.14.491	8:26	11:45	3:19	M,BP	Permanent	N	<1	SM	>50	CATTAILS, WILLOW, OSIER	C
18-VP-2	42.25.635	73.14.394	15:45	16:29	:44	LP	Temporary	N	<1	SM,SG	>50	LUSTRIEF, BUTTON BUSH	S
46-VP-5	42.22.934	73.14.824	11:35	12:04	:29	LP	Temporary	N	<1	SM	1-25	GRASSES, DOWNED TREE	S
18-VP-1	42.25.567	73.14.336	16:49	17:24	:35	LP	Temporary	N	<1	SM	26-50	GRASSES, LUSTRIEF	C
27-VP-1	42.24.728	73.14.060	13:08	17:40	4:32	LP	Permanent	N	<1	SM	1-25	OSIER,LUSTRIEF	S
27-VP-1 (W)	42.24.728	73.14.091	8:05	11:15	3:10	LP	Permanent	N	<1	SM	1-25	WILLOW, OSIER, GRASS, ALGAE	S
6.3	42.24.837	73.14.437	10:48	5:16	:37	LP	Temporary	N	<1	SM	26-50	LUSTRIEF	C
27B-VP-2	42.24.876	73.14.149	10:53	11:32	:39	LP	Temporary	N	<1	SG	0		S
40-VP-1	42.23.701	73.14.539	11:12	14:16	2:54	LP	Permanent	N	<1	SM	1-25		C
40-VP-3	42.23.730	73.14.499	9:54	12:20	2:26	LP	Permanent	N	<1	SM	26-50	SUB GRASSES, OSIER, LUSTRIEF	S
42-VP-1	42.23.595	73.14.616	13:19	16:10	2:51	LP	Temporary	N	<1	SM	>50	TRIANGLUAR GRASSES (DEAD), LUSTRIEF	C
58-A-VP-1	42.22.034	73.14.336	9:10	9:45	:35	M,B	Permanent	S	1-2	SM	26-50	PURPLE LUSTRIEA, GRASSES	C
8-VP-1	42.26.015	73.14.977	9:36	10:18	:42	LP	Temporary	N	<1	SM	1-25	OSIER	C
27B-VP-3	42.24.908	73.14.127	11:45	11:53	:08	LP	Temporary	N	<1	SG	0		S
33-VP-1	42.24.372	73.14.340	12:11	12:34	:23	LP	Temporary	N	<1	SM	1-25	OSIER, BRUSH	C
19-VP-6	42.25.216	73.14.456	14:05	18:15	4:10	LP	Permanent	N	1-2	SM	1-25	LUSTRIEF, WILLOWS, SUB-GRASS	S
27-VP-2	42.24.743	73.14.001	12:20	12:42	:22	LP	Temporary	N	<1	SM	1-25	OSIER,LIMBS	S
49A-VP-1	42.22.554	73.14.471	15:06	15:15	:09	LP, B	Temporary	N	<1	SM	26-50	OSIER, LUSTRIEF	C
42A-VP-1	42.23.403	73.14.717	8:10	10:56	2:46	LP	Permanent	N	1-2	SM	26-50	SUB GRASSES, LUSTRIEF	C
42-VP-3	42.23.546	73.14.578	9:37	9:49	:12	LP	Permanent	N	1-2	SM	0		S
23-VP-2	42.24.928	73.14.477	16:08	16:39	:31	LP	Permanent	N	<1	SM	1-25	OSIER,SUB-GRASSES,LUSTRIER	C
23-VP-2 (N)	42.24.954	73.14.492	16:52	17:15	:23	LP	Temporary	N	<1	SM	26-50	GRASSES, OSIER	C
40-VP-4	42.23.668	73.14.472	13:41/15:15	15:00/17:05	3:04	LP	Permanent	N	<1	SM	1-25	OSIER	S
40-VP-2	42.23.703	73.14.464	14:34	18:09	3:35	LP	Permanent	N	<1	SM	1-25		S
49-VP-1	42.22.464	73.14.441	13:54	14:45	:51	B	Permanent	S	>2	SM	1-25	LUSTRIEF	C
38-VP-1	42.23.879	73.14.226	11:18	11:55	:37	LP	Temporary	N	<1	SM	1-25	BUTTON BUSH, OSIER	S
38-VP-2(W)	42.23.819	73.14.365	10:25	12:03	1:38	LP,B	Permanent	S	1-2	SM	1-25	GRASSES	S
38-VP-2	42.23.841	73.14.307	9:34	10:08	:34	LP	Temporary	N	<1	SM	1-25	GRASSES	S
42-VP-2	42.23.539	73.14.654	9:57	10:44	:45	LP	Temporary	N	<1	SM	>50	LUSTRIEF, WILLOW	C
23-VP-1	42.24.892	73.14.499	15:30	15:35	:05	LP	Temporary	N	<1	SM	1-25	OSIER	C
38-VP-3	42.23.818	73.14.280	10:19	11:04	:45	LP	Temporary	N	<1	SM	1-25	BUTTON BUSH, OSIER	S
42-VP-4	42.23.451	73.14.704	10:54/15:35	12:50/16:39	3:00	LP	Permanent	S	1-2	SM	>50	LUSTRIEF, WILLOW	C
19-VP-5	42.25.291	73.14.350	14:12	15:35	1:23	LP	Temporary	N	1-2	SM	1-25	LUSTRIEF,OSIER	S
9.2	42.24.027	73.14.384	13:24	14:26	1:02	LP	Permanent	N	1-2	SM	1-25	LUSTRIEF, SUBGRASSES	C
8-VP-2	42.25.840	73.14.807	10:42	11:32	:50	LP	Temporary	N	<1	SM	1-25	OSIER,TREELIMBS	S
40A-VP-1	42.23.644	73.14.374	12:27	12:46	:19	LP	Temporary	N	<1	SM	1-25	LUSTRIEF, SUB-GRASSES	C
42-VP-5	42.23.438	73.14.617	16:48	18:15	1:27	LP	Temporary	N	<1	SM	26-50	LUSTRIFE, SUB GRASSES	C
19-VP-8	42.25.216	73.14.223	13:38	13:47	:09	LP	Temporary	N	<1	SM	1-25	SUB-GRASSES	C
4.7	42.25.198	73.14.405	14:05	14:45	:40	LP	Temporary	N	<1	SM	>50	LUSTRIEF	C

Notes:
d. LP = Lake/Pond, M = Marsh, BF = Bog/Fen, S = Stream, R = River Mainstem, B = Backwater, O = Oxbow, BP = Beaver Pond, SP = Spring
e. N = None, S = Slow, M = Moderate, F = Fast
f. SG = sand and gravel, SM = silt and mud
g. C = Clear, S = Stained

Table A-1 Survey Data

Survey Data Sheet Pond Name	Water Clarity [h]	Origin [i]	Drainage [j]	Frogs Calling	Frogs Seen	Adult *R. Pipiens* Sign	Average Conentration of tPCBs in sediment (mg/kg)	Number of *R. pipiens* Egg Masses	Fertilized Eggs Observed	Egg Development Progressing	Mass Density (per m^2)	Any *R. pipien* s Masses Present	Other Amphibian Masses Presnt	Other Adult Ambibians Present	Other Amphibian Sign Present	Fish Present
W-4	C	N	O	Yes	No	Yes	0.46	26	Yes	Yes	0.0033	Yes	Yes	No	Yes	No
W-4 (E)	CD	N	P	No	No	No	0.46	0	N/A	N/A	0	No	Yes	Yes	Yes	No
8-VP-4	C	N	N	No	No	No	0.47	2	Yes	Yes	0.0014	Yes	No	No	No	No
46-VP-4	C	N	N	No	No	No	0.53	0	N/A	N/A	0	No	No	No	No	No
46-VP-1	CD	N	P	No	No	No	0.76	0	N/A	N/A	0	No	No	No	No	No
23A-VP-1	C	N	N	No	Yes	Yes	0.90	2	Yes	Yes	0.00017	Yes	No	Yes	Yes	No
18-VP-2	C	N	N	No	No	No	1.1	0	N/A	N/A	0	No	No	Yes	Yes	No
46-VP-5	C	N	N	No	No	No	1.1	0	N/A	N/A	0	No	No	No	No	No
18-VP-1	C	N	N	No	No	No	1.7	0	N/A	N/A	0	No	No	Yes	Yes	No
27-VP-1	C	N	N	No	No	No	1.7	20	Yes	Yes	0.0044	Yes	Yes	Yes	Yes	Yes
27-VP-1 (W)	C	N	N	No	No	No	1.7	4	Yes	Yes	0.0015	Yes	Yes	Yes	Yes	Yes
6.3	C	N	N	Yes	Yes	Yes	2.0	0	N/A	N/A	0	No	No	No	No	No
27B-VP-2	C	N	N	No	No	No	4.1	0	N/A	N/A	0	No	Yes	Yes	Yes	No
40-VP-1	C	N	N	Yes	No	Yes	4.7	11	Yes	Yes	0.013	Yes	Yes	No	Yes	Yes
40-VP-3	C	N	N	Yes	No	Yes	4.7	5	Yes	No	0.0042	Yes	Yes	No	Yes	No
42-VP-1	C	N	N	Yes	No	Yes	7.5	15	Yes	Yes	0.0061	Yes	Yes	Yes	Yes	No
58-A-VP-1	C	N	O	No	No	No	8.4	0	N/A	N/A	0	No	No	No	No	No
8-VP-1	C	N	N	No	No	No	9.1	1	Yes	No	0.0042	Yes	No	No	No	No
27B-VP-3	C	N	N	No	No	No	10	0	N/A	N/A	0	No	Yes	Yes	Yes	No
33-VP-1	C	N	N	No	No	No	10	0	N/A	N/A	0	No	Yes	Yes	Yes	No
19-VP-6	C	N	N	Yes	Yes	Yes	12	55	Yes	Yes	0.014	Yes	No	Yes	Yes	Yes
27-VP-2	C	N	N	No	No	No	14	0	N/A	N/A	0	No	Yes	Yes	Yes	No
49A-VP-1	CD	N	O	No	No	No	15	0	N/A	N/A	0	No	No	No	No	No
42A-VP-1	C	N	O	No	Yes	Yes	16	0	N/A	N/A	0	No	No	No	No	Yes
42-VP-3	CD	N	N	No	No	No	19	0	N/A	N/A	0	No	No	No	No	No
23-VP-2	CD	N	O	No	No	No	19	0	N/A	N/A	0	No	No	Yes	Yes	No
23-VP-2 (N)	CD	N	N	No	No	No	19	0	N/A	N/A	0	No	No	Yes	Yes	No
40-VP-4	C	N	N	Yes	Yes	Yes	23	18	Yes	Yes	0.0090	Yes	Yes	No	Yes	Yes
40-VP-2	C	N	N	Yes	Yes	Yes	23	31	Yes	Yes	0.056	Yes	Yes	No	Yes	No
49-VP-1	CD	N	O	No	No	No	24	0	N/A	N/A	0	No	No	No	No	No
38-VP-1	C	N	N	No	No	No	29	0	N/A	N/A	0	No	Yes	Yes	Yes	No
38-VP-2(W)	C	N	O	Yes	Yes	Yes	21	8	Yes	Yes	0.0017	Yes	Yes	Yes	Yes	No
38-VP-2	C	N	N	No	No	No	32	0	N/A	N/A	0	No	Yes	Yes	Yes	No
42-VP-2	C	N	N	Yes	Yes	Yes	39	0	N/A	N/A	0	No	No	No	No	No
23-VP-1	C	N	N	No	No	No	45	0	N/A	N/A	0	No	No	Yes	Yes	No
38-VP-3	C	N	O	No	No	No	47	0	N/A	N/A	0	No	Yes	Yes	Yes	No
42-VP-4	C	N	O	Yes	Yes	Yes	48	7	Yes	Yes	0.0056	Yes	No	No	No	No
19-VP-5	CD	N	O	Yes	No	Yes	50	2	No	No	0.0017	Yes	No	Yes	Yes	No
9.2	CD	N	O	No	No	No	60	1	Yes	Yes	0.00057	Yes	No	No	No	No
8-VP-2	CD	N	N	No	No	No	61	0	N/A	N/A	0	No	No	No	No	No
40A-VP-1	CD	N	O	No	No	No	66	0	N/A	N/A	0	No	Yes	Yes	Yes	No
42-VP-5	CD	N	N	Yes	Yes	Yes	76	8	Yes	Yes	0.0051	Yes	No	No	No	Yes
19-VP-8	CD	N	O	No	No	No	105	0	N/A	N/A	0	No	No	Yes	Yes	No
4.7	C	N	O	No	Yes	Yes	230	0	N/A	N/A	0	No	No	Yes	Yes	No

Notes:
h. C = Clear, CD = Cloudy
i. N = Natural, C = Constructed
j. P = Permanent, O = Occasional, N = None
tPCBs = total polycholorinated biphenyls
N/A = not applicable

Table A-2 Water Pond Survey Comments

Site Name	Other Comments
8-VP-4	ADULT GREENS SEEN AND HEARD, TADPOLES IN POND ARE OLDEST TO DATE (R pipiens), 100s of older tadpoles
46-VP-4	VERY SMALL
46-VP-1	ONLY MADE 1 PASS (DID NOT SURVEY ALL OF BOTTOM), MANY SALAMANDER MASSES, WOOD FROG MASSES, COLD WATRE, ACIDIC, BEAVER PRESENT
23A-VP-1	SEARCHED OVER TWO DAYS, THOUSANDS, MILLIONS OF TADPOLES TRHOUGHOUT POND, NO LONGER AROUND EGG MASS AREAS, TADS LOOK LIKE LEPARD TADS STARTING TO SHOW SPECKLES, DIFFERENT HEAD TAIL NOW 12-15 MM, HEARD CODE 3 ON 4/18 4/20 HERE LATE
18-VP-2	SOME TADS-LARGER 2CM-QUICK, NO FISH, ADULT BULLS AND GREENS SEEN
46-VP-5	WOOD FROG MASSES, SPOTTED SALAMANDER MASSES, VERY TANIC WATER
18-VP-1	ADULT GREEN AND BULLS SEEN, HARD VISIBILITY
27-VP-1	SMALL FISH IN POND
27-VP-1 (W)	FISH IN POND
6.3	ALL LOT OF AREA COVERED W LYING DOWN LUSTRIEF, CLOSE TO 23A-VP-1
27B-VP-2	FEW SALAMNDER EGG MASSES, ADULTS GREENS SEEN, WOOD FROG MASSES
40-VP-1	WOOD FROG EGGS, SPOTTED SALAMANDER EGGS, 10 ' PICKEREL
40-VP-3	HUGE WOOD FROG COMMUNAL MASS-HATCHING, MANY SALAMANDER EGG MASSES
42-VP-1	LEPARD TADPOLES, ADULT GREENS HEARD
58-A-VP-1	100S OF NEWT SDALAMADERS, SPOTTED SALAMANDER EGG MASS, MOST NOT SURVEYED
8-VP-1	WOOD FROOG EGGS SEEN (SOME HATCHING), SALAMANDER MASS PRESENT
27B-VP-3	VERT TANNIC WATER
19-VP-6	MANY SMALL MINNOWS, BLUE GILLS, 24IN PIKE
27-VP-2	LOW WATER LEVELS, ADULT GRTEEN AND BULLS SEEN

Table A-2 Water Pond Survey Comments

Site Name	Other Comments
49A-VP-1	MANY SALAMANDER MASSES, MANY TADPOLES - 18 MM MORE COLORED
42A-VP-1	MIXED PATCHED OF LUSTRIEF AND BRUSH THROUGH THE POND AND AROUND ALL EDGES,MUTILPLE ADULT FISH (BULLHEAD,BASS, PIKE, SUNFISH), AM BITTERN AND VIRGINIA RAIL SEEN AT PONDS EDGE
42-VP-3	4 LARGE TADPOLES, CENTER NOT SURVEYED DUE TO SOFT MUD
23-VP-2	SHALLOW CHANELL
23-VP-2 (N)	MULTIPLE WOOD FROG MASSES, QUESTIONAL MASS(CORRECT SIZE AND COLOR BUT CONSISTENCY WAS MORE GELATANOUS, NOT COUNTED)
40-VP-4	2+ SUNFISH, NO WOOD FROG EGGS, NO SALAMANDER EGGS
40-VP-2	WOOD FROG EGGS PRESENT
49-VP-1	STOPPED HALFWAY DUE TO IMPASSIBLE, NUMEROUS SALAMANDER EGGS
38-VP-1	ADULT GREENS AND BULLS SEEN, MANY SALAMANDER MASSES SEEN, 1 WOOD FROG MASS
38-VP-2 (W)	MANY SALAMANDER MASSES
38-VP-2	MANY GREEN FROGS, WOOD FROG COLONY, SALAMADER EGG MASS
42-VP-2	
W-4	WOOD FROG MASSES, SPOTTED SALAMDER MASS
W-4 (E)	HARD TO SEARCH, AREA S OF YOKUM NOT SEARCHABLE,MORE WC DATA
23-VP-1	SMALL AND SHALLOW (8 IN), ONE PASS DOWN MIDDLE
38-VP-3	SALAMANDER EGG MASSES, WOOD FROG COMMUNAL MASSES, ADULT GREENS & BULLS SEEN & HEARD
42-VP-4	MANY TADPOLES, DEAD LUSTRIEF SUUROUNDS ENTIRE POND CARPOETING OVER SECTIONS THAT COULD NO LONGER BE SEARCHED, TWO MASSES WERE FOUND IN HOLES IN THE MAT.
19-VP-5	NO WOOD FROGG EGSS, NO SLAMANDR EGGS, ONE, QUESTIONABLE MASS
8-VP-2	2 WOOD FROG EGG MASSES

Table A-2 Water Pond Survey Comments

Site Name	Other Comments
40A-VP-1	DEAD ADULT TOAD, TOAD EGGS, ADULT GREEN HEARD
42-VP-5	SURVEYED OVER TWO DAYS, EMPTY WHITE EGG MASSES, SEVERAL TADPOLES, 1 FISH SEEN
19-VP-8	VERY SMALL, TOAD EGGS, GREEN SEEN
4.7	ADULT GREENS AND BULLS SEEN, ONE SECTION TOO THICK TO SEARCH
33-VP-1	ADULT GREENS SEEN, MANY OLDER TADS
9.2	SOME TADPOLES DISPERSED THROUGH THE POND, 1 LAP ONLY
6.7	TOO MUDDY TO SURVEY, KM SUNK IN OVER WADERS. DID NOT CHECK CATTAILS